DOI:10.1046/j.1365-2125.2003.02033.x

Clinical inhibition of CYP2D6-catalysed metabolism by the antianginal agent perhexiline

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Keywords
CYP2D6, dextromethorphan, genotype, metabolic ratio, perhexiline

Received
15 July 2003
Accepted
16 October 2003

Aims
Perhexiline is an antianginal agent that displays both saturable and polymorphic metabolism via CYP2D6. The aim of this study was to determine whether perhexiline produces clinically significant inhibition of CYP2D6-catalysed metabolism in angina patients.

Methods
The effects of perhexiline on CYP2D6-catalysed metabolism were investigated by comparing urinary total dextrorphan/dextromethorphan metabolic ratios following a single dose of dextromethorphan (16.4 mg) in eight matched control patients not taking perhexiline and 24 patients taking perhexiline. All of the patients taking perhexiline had blood drawn for *CYP2D6* genotyping as well as to measure plasma perhexiline and *cis*-OH-perhexiline concentrations.

Results
Median (range) dextrorphan/dextromethorphan metabolic ratios were significantly higher ($P < 0.0001$) in control patients, 271.1 (40.3–686.1), compared with perhexiline-treated patients, 5.0 (0.3–107.9). In the perhexiline-treated group 10/24 patients had metabolic ratios consistent with poor metabolizer phenotypes; however, none was a genotypic poor metabolizer. Interestingly, 89% of patients who had phenocopied to poor metabolizers had only one functional *CYP2D6* gene. There was a significant negative linear correlation between the log of the dextrorphan/dextromethorphan metabolic ratio and plasma perhexiline concentrations ($r^2 = 0.69$, $P < 0.0001$). Compared with patients with at least two functional *CYP2D6* genes, those with one functional gene were on similar perhexiline dosage regimens but had significantly higher plasma perhexiline concentrations, 0.73 (0.21–1.00) *vs.* 0.36 (0.04–0.69) mg l^{-1} ($P = 0.04$), lower *cis*-OH-perhexiline/perhexiline ratios, 2.85 (0.35–6.10) *vs.* 6.51 (1.84–11.67) ($P = 0.03$), and lower dextrorphan/dextromethorphan metabolic ratios, 2.51 (0.33–39.56) *vs.* 11.80 (2.90–36.93) ($P = 0.005$).

Conclusions
Perhexiline significantly inhibits CYP2D6-catalysed metabolism in angina patients. The plasma *cis*-OH-perhexiline/perhexiline ratio may help to both phenotype patients and predict those in whom perhexiline may be most likely to cause clinically significant metabolic inhibition.

Introduction

Perhexiline [2-(2,2-dicyclohexylethyl)piperidine], is a prophylactic antianginal agent that is thought to improve myocardial efficiency by modifying myocardial energy metabolism [1, 2]. While this mechanism of action ensures freedom from negative inotropic effects, high plasma concentrations of perhexiline have the potential over long periods of time to induce the development of phospholipidoses with severe hepatic and neurological toxicity [3–5]. The clinical use of perhexiline has therefore been limited to patients with intractable angina who are refractory or intolerant to conventional therapy, or who are unsuitable for surgical treatment. Maintaining plasma perhexiline concentrations between 0.15 and 0.60 mg l^{-1} (0.5–2.1 μM) has been shown to minimize the risk of clinically significant long-term toxicity without significantly compromising clinical efficacy [6, 7]. Thus, therapeutic drug monitoring is necessary to guide perhexiline dosage, since as a result of polymorphic [8, 9] and saturable [10, 11] metabolism there is no clear relationship between dose and steady-state plasma perhexiline concentrations [12, 13]. More recently, perhexiline has also increasingly been used as a component of short-term therapy in patients with unstable angina pectoris, as plasma perhexiline concentrations between 0.15 and 0.60 mg l^{-1} have been associated with potentiation of platelet responsiveness to nitric oxide, which in turn is associated with suppression of anginal symptoms [14, 15].

The primary mechanism of perhexiline clearance in humans appears to be the CYP2D6-catalysed formation of *cis*-OH-perhexiline [12]. We have previously demonstrated that the plasma concentration ratio of *cis*-OH-perhexiline/perhexiline may be used to phenotype patients with respect to CYP2D6 metabolizer status, and that CYP2D6 phenotype is a major determinant of perhexiline dose, which can range from 100 mg $week^{-1}$ in CYP2D6 poor metabolizers to 500 mg day^{-1} in phenotypic ultra-rapid metabolizers [12]. Given the major role of CYP2D6 in perhexiline clearance, patients may also be subject to a number of important drug–drug interactions, such as the inhibition of perhexiline clearance by a number of selective serotonin reuptake inhibitors [16]. To date, the ability of perhexiline to inhibit the CYP2D6-catalysed metabolism of other coadministered drugs has not been investigated. However, perhexiline is likely to be a clinically significant competitive inhibitor of CYP2D6, since its K_m for metabolism by human liver microsomes (3.3 ± 1.5 μM) [8] and its K_i for competitive inhibition of dextromethorphan metabolism by human liver microsomes (0.4 μM) [17] are both within the range of plasma perhexiline concentrations attained clinically.

The *O*-demethylation of dextromethorphan to dextrorphan is catalysed by CYP2D6, and dextromethorphan is a well-established probe drug for phenotyping patients with respect to CYP2D6 metabolizer status, usually on the basis of the metabolic ratio of urinary dextrorphan/dextromethorphan concentrations [18–21]. The aim of this study was to investigate whether perhexiline produces clinically significant inhibition of CYP2D6-catalysed metabolism by comparing the dextrorphan/dextromethorphan metabolic ratios following a single dose of dextromethorphan in two groups of patients with chronic or acute ischaemic heat disease, a control group not taking perhexiline, and a second group taking perhexiline.

Methods

Chemicals

Dextromethorphan, dextrorphan D-tartrate, and perhexiline maleate were purchased from Sigma Chemical Co. (St Louis, MO, USA). Pholcodine was obtained from F. H. Faulding (Adelaide, Australia). *Cis*-OH-perhexiline was a gift from Marion Merrell Dow Inc. (Cincinnati, OH, USA). All other reagents were of analytical grade.

Patients

This study was approved by the Ethics of Human Research Committee of The Queen Elizabeth Hospital. Patients admitted to the Cardiology Unit of The Queen Elizabeth Hospital gave written informed consent prior to participating in the study. All patients had a diagnosis of stable or unstable angina pectoris or acute myocardial infarction, and were not taking any medications known to cause clinically significant inhibition of CYP2D6-catalysed metabolism (Table 1). Patients taking β-blockers were not excluded from the study because, although a number of β-blockers are substrates for CYP2D6, to our knowledge they have not been reported to cause clinically significant inhibition of CYP2D6. Of the 24 patients in the perhexiline-treated group, 19 had been diagnosed with unstable angina or acute myocardial infarction, and 13 had been taking perhexiline for <2 weeks prior to the study. Patients in the control group were not taking perhexiline, but were matched with respect to other medications, age and hepatic and renal functions. All perhexiline-treated subjects had one blood sample drawn for *CYP2D6* genotyping and a second blood sample to determine trough plasma perhexiline and *cis*-OH-perhexiline concentrations. The genotyping specimen was collected in EDTA anticoagulant and stored at 4 °C until analysis (up to 6 months). The other sample was immediately centrifuged and the plasma

Table 1
Medications taken by the perhexiline-treated and control patients

	Perhexiline (n = 24)	Control (n = 8)
Anticoagulants/antithrombotics (aspirin, enoxaparin, clopidogrel, warfarin)	23 (96%)	7 (88%)
Nitrates (isosorbide dinitrate, isosorbide mononitrate)	20 (83%)	8 (100%)
β-blockers (atenolol, carvedilol, metoprolol, sotalol)	10 (42%)	4 (50%)
ACE inhibitors (enalapril, fosinopril, lisinopril, omapatrilat, perindopril, ramipril, trandolapril)	12 (50%)	3 (10%)
Angiotensin-II receptor antagonists (irbesartan, telmisartan)	4 (17%)	2 (25%)
Ca^{2+}-channel blockers (diltiazem, felodipine, verapamil)	10 (42%)	4 (50%)
Statins (atorvastatin, fluvastatin, simvastatin)	15 (63%)	4 (50%)
Diuretics (frusemide, spironolactone, metolazone)	11 (46%)	1 (13%)
Hypoglycaemics (gliclazide, glimepiride, insulin, metformin)	7 (29%)	5 (63%)
Bronchodilatators (beclomethasone, ipratropium bromide, salbutamol)	3 (13%)	0 (0%)
Others (allopurinol, ciprofloxacin, codeine, colchicine, digoxin, doxepin, gemfibrozil, gentamicin, lansoprazole, lanoprost, norfloxacin, omeprazole, pantoprazole, paracetamol, penicillin, prazosin, ranitidine, sorbitol, sulfasalazine, temazepam, thyroxine, trimethoprim)	18 (75%)	4 (50%)

was stored at −20 °C until analysis. All patients were asked to empty their bladder prior to receiving 10 ml of cough mixture containing 16.4 mg dextromethorphan (20 mg dextromethorphan hydrobromide, Tussinol; Pfizer Pty. Ltd, NSW, Australia), and subsequently all urine was collected for the following 8 h. The total volume of urine collected was noted, urine pH was measured and three 10-ml aliquots were stored at −20 °C until analysis. A 10-ml aliquot of predose (blank) urine was similarly stored at −20 °C.

Plasma concentrations of perhexiline and *cis*-OH-perhexiline were measured by high-performance liquid chromatography (HPLC) as previously described [12]. The concentrations of dextromethorphan and dextrorphan in urine were measured, both with and without β-glucuronidase hydrolysis, using an established HPLC method [22]. The lower limits of quantification for dextrorphan and dextromethorphan were 0.1 and 0.02 mg l^{-1}, respectively. At these concentrations the intra-assay coefficients of variation and biases were 1.9% and 6.6%, respectively, for dextrorphan, and 3.0% and 13.3% for dextromethorphan. The upper limits of quantification for dextrorphan and dextromethorphan were 20 and 5 mg l^{-1}, respectively, with intra-assay coefficients of variation and biases <5%.

Genotyping

Genomic DNA was isolated from blood samples using a QIAamp DNA mini kit according to the manufacturer's protocol (Qiagen Pty Ltd, Clifton Hill, Australia). Genotyping for the detection of *CYP2D6*1* to **4* and **6* to **9* was performed using a new sequencing method [23]. In brief, initial polymerase chain reactions (PCRs) using primers previously published by Heim and Meyer [24] were performed to produce two templates isolating regions in exons 3–4, position 1297–2034; and exons 5–6, position 2010–3112 (numbers based on translation start). PCR products were purified using a QIAquick PCR purification kit (Qiagen) and sequencing reactions were performed with an ABI PRISM® BigDye™ Terminator kit version 3 according to the manufacturer's protocol, with analysis on an ABI PRISM® 3700 DNA analyser (Applied Biosystems, Scoresby, Victoria, Australia). Genotyping for the detection of *CYP2D6*5* and **16,* and *CYP2D6* gene duplication (*CYP2D6*1xN*, *CYP2D6*2xN* and *CYP2D6*4xN*) was performed as described previously [25, 26]. The gene duplication assay was validated using DNA samples kindly provided by Dr U. Griese (Dr Margarete Fischer-Bosch Institute of Clinical Pharmacology, Stuttgart, Germany) previously identified as carrying duplicated copies of the *CYP2D6* gene. In addition, each assay was run with a positive control sample and false negatives were avoided with the amplification of a control band for each patient sample. However, as this method does not distinguish which *CYP2D6* allele carries the duplication, samples with duplicated *CYP2D6* gene copies will be referred to as *CYP2D6(*x/*y)xN*, where *x* and *y* represent the classification of the single allele and *xN* signifies duplication.

Statistical analysis

All dextromethorphan, dextrorphan, perhexiline and *cis*-OH-perhexiline data are presented as median (range)

values, with between-group comparisons carried out using a one-tailed Mann–Whitney *U*-test (GraphPad Software Inc., San Diego, CA, USA). Patients' demographic data are presented as mean (SD) with comparisons between control and perhexiline-treated patients' gender carried out using a Fisher's exact test, and using a two-tailed *t*-test to compare age, urine pH, plasma creatinine, hepatic and renal function. *P*-values <0.05 were considered to be statistically significant.

Results

A total of eight patients were recruited in the control group and 24 in the perhexiline-treated group. As shown in Table 2, the two groups of patients were comparable with respect to age, gender distribution, renal and hepatic function. In the perhexiline-treated group, four patients had alanine aminotransferase results above the normal range and one had an aspartate aminotransferase result above the normal range. Previous work has shown that whilst liver dysfunction causes some impairment of dextromethorphan *O*-demethylation, it is not sufficient to cause incorrect assignment of phenotype [27], and these patients were not excluded from the study. All patients were on polytherapy, with control subjects taking an average of six medications, and perhexiline-treated patients an average of seven other medications in addition to perhexiline.

The perhexiline-treated patients, most of whom had been taking perhexiline for <2 weeks, had trough plasma perhexiline concentrations ranging from 0.06 to 1.11 mg l^{-1}, *cis*-OH-perhexiline concentrations of 0.31–3.68 mg l^{-1}, and ratios of *cis*-OH-perhexiline/perhexiline of 0.35–21.11. For the 13 patients who had only recently commenced treatment with perhexiline, the plasma perhexiline and *cis*-OH-perhexiline concentrations represented the first measured concentrations, which were subsequently used for routine dose titration.

The median (range) 0–8-h urinary molar metabolic ratio of total (unchanged +conjugated) dextrorphan/dextromethorphan was significantly higher ($P < 0.0001$ Mann–Whitney *U*-test) in the control patients, 271.1 (40.25–686.1), compared with patients treated with perhexiline, 5.02 (0.33–107.9) (Figure 1). The metabolic

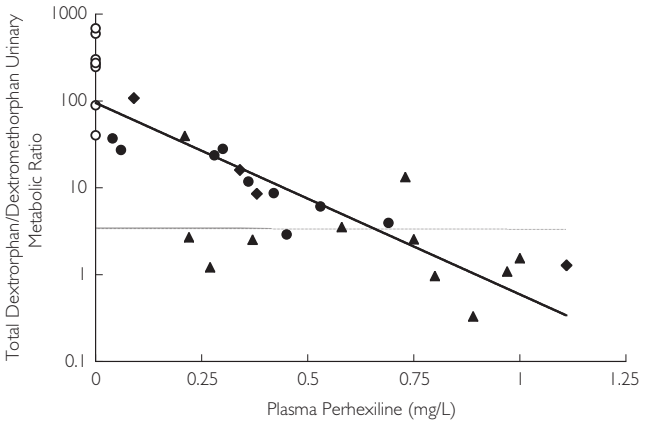

Figure 1
Linear correlation between plasma perhexiline concentrations and the log of total dextrorphan/dextromethorphan urinary metabolic ratios in eight control (open symbols) and 24 perhexiline-treated (closed symbols) patients ($r^2 = 0.692$, $P < 0.0001$). For the perhexiline-treated patients *CYP2D6* genotype is indicated as (▲) one active gene, (●) two or more active genes, or (◆) unknown. The dotted line indicates the antimode separating poor metabolizers and extensive metabolizers [18]

Table 2
Physiological characteristics of the perhexiline-treated and control patients

	Perhexiline	Control
Males : females	15 : 9	7 : 1
Age (years)	71.9 (10.6)	68.6 (8.3)
Plasma creatinine (mmol l^{-1})	0.151 (0.061)	0.121 (0.041)
Albumin (g l^{-1})	40 (4)	41 (3)
Total bilirubin (μmol l^{-1})	11 (4)	8 (6)
GGT (Gamma-glutamyltransferase) (U l^{-1})	50 (38)	27 (13)
ALP (Alkaline phosphatase) (U l^{-1})	94 (31)	94 (15)
Alanine aminotransferase (U l^{-1})	45 (42)	25 (10)
Aspartate aminotransferase (U l^{-1})	38 (18)	17 (4)
Urine pH	5.8 (0.9)	5.4 (0.7)

Data are shown as mean (SD). There were no statistically significant differences between control and perhexiline-treated groups ($P > 0.05$, Fisher's exact test for gender comparison, $P > 0.05$ unpaired t-test all other comparisons).

Table 3
Genotype, median (range) plasma perhexiline concentrations (Px), plasma concentration ratio of *cis*-OH-perhexiline (OHPx)/Px and urinary total dextrorphan (DR)/dextromethorphan (DM) metabolic ratio in the perhexiline-treated patients

No. of functional genes	Genotype		Px (mg l^{-1})	OHPx/Px	DR/DM
1	*CYP2D6*1/*4*	(*n* = 5)	0.73	2.85	2.51
	*CYP2D6*1/*5*	(*n* = 1)	(0.21–1.00)	(0.35–6.10)	(0.33–39.56)
	*CYP2D6*2/*3*	(*n* = 1)			
	*CYP2D6*2/*4*	(*n* = 3)			
	*CYP2D6*1/*6*	(*n* = 1)			
≥ 2	*CYP2D6*1/*1*	(*n* = 1)	0.36†	6.51†	11.80†
	*CYP2D6*1/*2*	(*n* = 4)	(0.04–0.69)	(1.84–11.67)	(2.90–36.93)
	*CYP2D6*2/*2*	(*n* = 1)			
	*CYP2D6(*1/*1)xN*	(*n* = 1)			
	*CYP2D6(*1/*2)xN*	(*n* = 1)			
	*CYP2D6(*2/*2)xN*	(*n* = 1)			

†$P < 0.05$ compared with patients with one functional allele (one-tailed Mann–Whitney U-test).

ratios observed in the control patients were consistent with extensive metabolizer phenotypes [18]. For the perhexiline-treated group, 10/24 patients had metabolic ratios consistent with poor metabolizer phenotypes [18], only one of which had elevated liver function tests. There was a significant ($P < 0.0001$) negative linear correlation between plasma perhexiline concentrations and the log of total dextrorphan/dextromethorphan metabolic ratio (Figure 1). In the control patients there was a significant linear correlation between urine pH and the total dextrorphan/dextromethorphan metabolic ratio ($r^2 = 0.8413$, $P = 0.0013$). However, there was no relationship between urine pH and total dextrorphan/dextromethorphan metabolic ratio in the perhexiline-treated patients.

The median (range) total recovery of the oral dextromethorphan dose in urine was 20.4% (5.8–45.7) and 5.7% (0.8–41.1) in control and perhexiline-treated patients, respectively, and was similar to previous reports [21]. The total dose recovered was less ($P < 0.05$) in perhexiline-treated patients compared with controls, primarily as a result of lower ($P < 0.01$) recoveries of total dextrorphan in perhexiline-treated patients (median = 4.0%, range 0.6–40.8%) compared with controls (median = 20.3%, range 5.8–45.7%). The dextrorphan recovered in urine was present primarily as the glucuronide conjugate, which accounted for 96.7% (93.0–99.1%) and 95.5% (85.4–99.5%) of the total dextrorphan excreted in control and perhexiline-treated patients, respectively ($P > 0.05$). In contrast, the dextromethorphan recovered in urine was present entirely as unconjugated drug, accounting for 0.07% (0.03–0.19%) and 0.67% (0.03–4.99%) of the dose in control and perhexiline-treated patients, respectively ($P < 0.001$).

Genotyping results for 20 of the 24 perhexiline-treated patients are shown in Table 3, indicating that none of the patients was a genotypically poor metabolizer. Three patients had more than two functional *CYP2D6* genes, six patients had two functional genes, and 11 patients only one functional gene. Despite both groups being on similar dosage regimens of perhexiline ($P > 0.05$, data not shown), there were significant differences in plasma perhexiline concentrations, plasma *cis*-OH-perhexiline/perhexiline concentration ratios and urine dextrorphan/dextromethorphan metabolic ratios between patients with at least two functional genes and those with one functional gene (Table 3).

Discussion

The urinary metabolic ratio of total (unchanged +conjugated) dextrorphan/dextromethorphan is strongly correlated with the partial clearance of dextromethorphan by CYP2D6-catalysed *O*-demethylation to dextrorphan [28]. Consistent with the important role of CYP2D6 in the formation of dextrorphan, there is a bimodal distribution in the metabolic ratio of subjects phenotyped with dextromethorphan, with an antimode at the molar dextromethorphan/total dextrorphan urinary metabolic ratio of 0.3 separating poor metabolizers (ratio >0.3) from the rest of the population [18]. Importantly, in the absence of drugs known to inhibit

CYP2D6-catalysed metabolism, the antimode of 0.3 has been shown to separate genotypically classified poor metabolizers from the rest of the population [19–21].

In this study, 32 patients with symptomatic myocardial ischaemia were phenotyped with dextromethorphan. Of these patients, the eight controls, who were not taking perhexiline, all had metabolic ratios within the ranges previously reported for extensive metabolizers. In contrast, the 24 patients who were also taking perhexiline had significantly lower total dextrorphan/dextromethorphan metabolic ratios compared with the controls, and 10 (42%) had ratios consistent with poor metabolizers (Figure 1). The frequency of poor metabolisers in a Caucasian population is approximately 7% [19]. Thus, the high proportion of apparent poor metabolizers in the perhexiline-treated group is suggestive of phenocopying. Indeed, none of the patients taking perhexiline was identified as a poor metabolizer by genotyping or, other than perhexiline, was taking any drugs known to inhibit CYP2D6-catalysed metabolism (Table 1). The two groups of patients were matched with respect to hepatic and renal function (Table 2) as well as urine pH, which can affect the renal tubular reabsorption of drugs and has previously been shown to affect the dextrorphan/dextromethorphan metabolic ratio [29]. Thus, the observed differences in metabolic ratio between control and perhexiline-treated patients were unlikely to have been due to differences in overall hepatic function or in the renal clearances of dextromethorphan, dextrorphan or dextrorphan-glucuronide, and it was more likely that perhexiline itself affected the metabolism of dextromethorphan.

The significant negative linear correlation between plasma perhexiline concentrations and the log of total dextrorphan/dextromethorphan metabolic ratio further supports the inhibition of CYP2D6 by perhexiline (Figure 1). Importantly, the difference in dextrorphan/dextromethorphan metabolic ratios between control and perhexiline-treated patients remained statistically significant ($P < 0.0001$) even when only the patients with perhexiline concentrations within the therapeutic range ($n = 13$) were considered. Thus, this study demonstrates that inhibition of CYP2D6 by perhexiline is dependent upon its concentration in plasma and occurs even when perhexiline concentrations are within the recommended therapeutic range, consistent with the previously reported K_i value of 0.4 μM for perhexiline's inhibition of dextromethorphan metabolism in human liver microsomes [17].

Several drugs have been shown to cause clinically significant inhibition of CYP2D6-catalysed metabolism, including fluoxetine, paroxetine, quinidine, methadone and dextropropoxyphene [28, 30–33]. Both fluoxetine and paroxetine have been shown to increase urinary dextromethorphan/dextrorphan metabolic ratios significantly, with 42% and 83% of subjects, respectively, phenocopied to poor metabolizers [30]. In our study, 42% of patients treated with perhexiline phenocopied to poor metabolizers, suggesting that the inhibition of CYP2D6-catalysed metabolism produced by perhexiline was similar to that produced by fluoxetine.

Even in the absence of any inhibitors of CYP2D6, the dextrorphan/dextromethorphan urinary metabolic ratios in a genotypic extensive metabolizer population vary by as much as 1000-fold [19–21]. This variability is due largely to differences in the number of active *CYP2D6* genes individuals inherit which appear to determine the relative expression and liver concentrations of CYP2D6 protein [34]. Thus, compared with patients with at least two functional genes, patients with only one functional *CYP2D6* gene are likely to have a lower capacity for CYP2D6-catalysed metabolism. These patients should therefore attain higher plasma perhexiline concentrations and be more likely to display clinically significant inhibition of CYP2D6-catalysed metabolism by perhexiline or other coadministered drugs. Indeed, in this study 89% of the patients who phenocopied to poor metabolizers had only one functional *CYP2D6* gene, whereas 73% of the patients who were not phenocopied had at least two functional *CYP2D6* genes (Figure 1). In addition, whilst the patients with only one functional gene were on similar doses of perhexiline, they had significantly higher plasma perhexiline concentrations and lower *cis*-OH-perhexiline/perhexiline ratios, compared with the patients with at least two functional genes (Table 3).

It has been estimated that approximately 25% of clinically used medications are metabolized to some extent by CYP2D6 [35]. The patients in whom perhexiline is used are typically elderly and on polytherapy. Thus, clinical inhibition of CYP2D6-catalysed metabolism by perhexiline should be considered in order to minimize undesired clinical drug–drug interactions. We have previously reported that the plasma *cis*-OH-perhexiline/perhexiline concentration ratio may be a convenient means of phenotyping patients, with ratios <0.3 being indicative of CYP2D6 poor metabolizers [12]. The observed difference in this ratio between extensive metabolizers with one or two functional alleles further suggests that it may also be a useful measure of relative CYP2D6 metabolic capacity in this subgroup with low extensive metabolizer ratios; such patients may require lower doses of perhexiline and may be more suscep-

tible to CYP2D6-mediated drug–drug interactions in general.

This work was presented, in part, at the Australian Health and Medical Research Congress, Melbourne, Australia, 2002. B.J.L.D. was the recipient of a Queen Elizabeth Hospital Research Foundation Honours Research Scholarship. J.K.C. is the recipient of a CJ Martin Fellowship from the National Health and Medical Research Council of Australia. We gratefully acknowledge funding for the PCR equipment from the Faculty of Health Sciences, The University of Adelaide.

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